REMARKS

Claims 5-16 have been withdrawn from consideration. As such, claims 1-4 and 17-19 are pending.

Rejections under 35 U.S.C. §112, first paragraph

Claims 17-19 have been rejected under 35 U.S.C. §112, first paragraph with the assertion that the specification only supports artificial SH3 domains derived from Hck-SH3 and targeted to the HIV-I Nef protein. Claim 17 has been amended to define the sequences as being derived from Hck-SH3 and targeted to the HIV-1 Nef protein. Withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §112, second paragraph

Claims 1-4 have been rejected under 35 U.S.C. §112, second paragraph as being indefinite.

More specifically, claim 1 has been rejected as being indefinite for failing to recite the identification of the SH3 domains. Claim 1 has been amended as suggested by the Examiner. As such, withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. §102(b)/103

Claims 1-4 remain rejected under 35 U.S.C. §102(b) or §103 as being anticipated by or obvious over Lee et al. (EMBO Journal). In response to Applicants' amendments and remarks of

September 17, 2001, the Examiner asserts that Lee et al. is not limited to the Fyn SH3 RT-loop and extends to multiple SH3 kinase domains, which contain multiple mutant RT-loops. The Examiner further asserts that making an SH3 which differs from the wild SH3 region of a kinase constitutes a "new sequence" of the reference.

Lee et al. is further asserted to teach and suggest making artificial SH3 domains of different kinases that contain random substitutions within the non-conserved regions of the RT-loop region. Thus, the basis of the present rejection is the Examiner's interpretation of Lee et al. versus Applicants' interpretation of the teachings of the reference.

Submitted herewith is a signed Declaration submitted under 35 U.S.C. §1.132, of Dr. Kalle SAKSELA, who is an author of the Lee et al. article. Dr. SAKSELA states, in part, in the declaration,

> In the Lee et al. article, we showed that the RT loop of SH3 is important with respect to binding specificity. Namely, when one, two or three amino acids (most notably the Ile residue 96) identical or similar to those found in the Hck tyrosine kinase, were transferred into the corresponding position in the RT-loop of the Fyn tyrosine kinase, the binding properties of Fyn (i.e. ability to bind to HIV Nef) could be made to resemble those of Hck.
>
> With the work done in Lee et al., we did not create totally new binding properties, rather we merely mimicked what already exists in nature. In other words, when Fyn was made structurally similar to Hck, its function, i.e. binding properties became that of Hck. Thus, the important and sole teaching of Lee et al. is that the more closely the amino acid sequence in the RT-loop of one SH3 domain is made to resemble that of

another SH3 domain, the more similar will their binding specificities become. It is critical to note that Lee et al. did not suggest or even hint that an artificial SH3 domain able to bind to HIV Nef could be made by any other way than by mimicking the RT-loop sequence of the Hck tyrosine kinase. In fact, based on the teachings of Lee at al. this would seem unlikely.

By contrast, the present invention discloses that specific binding to HIV Nef can be achieved by combinations of six RT-loop amino acids that bear no similarity whatsoever with the corresponding sequence in Hck or in any other known natural SH3 domain.

In other words, the amino acid sequences of the RT loops of the artificial SH3 domains as produced in the present invention, which sequences bind strongly to HIV-1 Nef protein, do not reflect any naturally occurring amino acid sequences and therefore cannot be achieved on the basis of the existing prior art, including the work we did in Lee et al. It should be noted that in nature only a limited number of SH3 domains exist (the human genome appears to encode about 200 different domains).

Thus by mimicking nature, as we did in Lee et al., the amount of naturally occurring domains is the maximum limit of different binding specificities that may be obtained. On the other hand, by using an artificial RRT-SH3 library of the present invention, we have about achieved 64 million different SH3 binding domains, and thus the present invention provides an almost unlimited source of new binding properties.

Another key aspect and advantage of the invention is that one can obtain <u>unnaturally</u> high binding affinities as measured by the strength of binding, which is never possible by mimicking nature, as with Lee et al. With the present invention, artificial SH3 domains can be produced which can displace the natural SH3-mediated protein interactions, which will be useful in both experimental and therapeutic applications. The ability to obtain extremely high binding affinities is not possible from Lee et al.

The Examiner is of the opinion that modification of the Fyn-SH3 to mimic Hck is a "general example" and that based on that example many different SH3 domains could be modified in various

ways. However, the Lee et al. article only describes how the binding capacity of one known SH3 domain can be changed to the binding capacity of another known SH3 domain. Consequently, although the FynR961 of Lee et al. is "artificial" in the sense that the protein is different from wild-type Fyn-SH3, the isoleucine at this position is copied directly from nature. Thus, while FynR961 may be termed "artificial" the SH3 domain is not, in fact, artificial, because it occurs in nature, just not in the context of Fyn. Lee et al. fails to disclose or suggest any modification of Hck or any other SH3 domain other than by modifying the Hck to mimic other known, naturally occurring SH3 domains.

The Examiner also is of the position that Lee et al. describes the preparation of an artificial SH3 domain that can have "random" amino acids. However, as discussed by Dr. Saksela, who is an author of Lee et al., the Examiner has misinterpreted the reference. There is no disclosure of randomization in Lee et al. "Randomization" means the creation of totally new peptides having one or more amino acid substitutions. Lee et al. merely describe a study of how Fyn and Hck are different from each other with regard their respective binding to Nef protein and how Fyn can be deliberately, i.e. not randomly, improved by using Hck as a model.

The Examiner states that Lee et al. uses recombinant libraries. However, the Examiner has again misinterpreted the

reference. In Lee et al., a small number of recombinant plasmids were used, which encoded proteins whose ability to bind Nef protein had been tested, one at a time. The set of plasmids used in Lee et al. is not a "library" nor would it considered a "library" by one skilled in the art, applying the commonly accepted meaning for this term. A "library" is made of a great number of recombinant vectors, from which the best or most appropriate is selected. The present invention exploits the SH3 library created by the inventors. Lee et al. does not use a library nor randomization. The designed and specifically directed mutagenesis of Lee et al. is an approach that is completely the opposite of the randomized library of the invention. Thus, contrary to the assertion of the Examiner, the present invention is neither disclosed nor suggested by Lee et al. and withdrawal of the rejection is respectfully requested.

Claims 1-4 and 17-19 have been further rejected under 35 U.S.C. §103(a) as being obvious over Lee et al. combined with Sparks et al. Lee et al. is asserted to differ from the present invention only in failing to explicitly teach the generation of "artificial Hck-SH3" libraries by randomizing the non-conserved hexapeptide 69-74 RT-loop sequence of Hck to obtain completely random libraries comprising 20^6 artificial Hck-SH3 proteins differing from the wild-type at hexapeptide 69-74 for subsequent ligand screening. Lee et al. is further relied upon for

providing motivation to make recombinant libraries from randomization of the non-conserved (e.g. variable RT loop) hexapeptide 69-74 of Hck or the corresponding region of Src kinase. Sparks et al. is asserted to teach the use of "biased peptide libraries" or "random peptide libraries" as a means for making and screening Src SH3 ligands, thus providing additional motivation. The Examiner asserts that it would be obvious from the teachings of Lee et al. and Sparks et al. to generate "artificial Hck-SH3" libraries by making random peptide libraries having the hexapeptide 69-74. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

As discussed above and in the Declaration of Dr. SAKSELA, Lee et al. disclose the use of targeted mutagenesis to specifically change amino acids to copy the SH3 domains of other naturally occurring proteins. With the present invention randomized libraries were used to create completely new SH3 domains with new binding properties. Thus, Lee et al. fails to teach or suggest the present invention. The deficiencies of Lee et al. are not overcome by the combination with Sparks et al.

Sparks et al. discloses the use of the phage-display method to modify short, linear peptides containing the PXXP motif, in order to enable the peptide to bind as efficiently as possible to particular naturally occurring SH3 domains, for example the SH3 domain of Src. Sparks et al. discloses the conventional use of phage-display for peptide modification, whereas the present

invention uses phage-display to modify a region and determine the binding specificity of a native, globular protein structure (SH3 domain).

The Examiner asserts that the invention is obvious over Sparks et al. combined with Lee et al. however, the teachings of Lee et al. and Sparks et al. are in opposite to each other.

Sparks et al. report that by varying the two amino acids flanking the PXXP motif (which is the linear proline-rich, SH3 binding sequence of SH3 target proteins), one can substantially affect the ability of SH3 domains to identify the target peptides. The study of Sparks et al. supports the accepted understanding in the art that the molecular interactions between the amino acids of the PXXP motif and the cognate amino acid residues of the SH3 domains determine strength and specificity of binding between the SH3 domain and its ligands.

Lee et al., on the other hand, teach that the Fyn-SH3 domain can be modified to resemble Hck-SH3 in terms of the binding properties by replacing the variable region of the RT-loop with the corresponding Hck-SH3 region. However, the RT-loop does not participate in the molecular interactions of the PXXP motif. Thus, the teachings of Lee et al. and Sparks et al. contradict each other with what they disclose as being important regions for binding. As such, one skilled in the art would not be able to achieve the present invention by combining Lee et al. with Sparks et al. and the present invention is therefore not obvious over

the references. Withdrawal of the rejection is respectfully requested.

As the above-indicated amendments and remarks address and overcome the rejections of the claims, withdrawal of the rejections and allowance of the claims are respectfully requested.

Should the Examiner have any questions regarding the present application she is requested to please contact MaryAnne Armstrong, PhD (Reg. No. 40,069) in the Washington DC area at (703) 205-8000.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) respectfully petition(s) for a one (1) month extension of time for filing a reply in connection with the present application, and the required fee of $55.00 is attached hereto.

A marked up version showing amendments to the specification and claims is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §1.16 or under 37 C.F.R. §1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

Gerald M. Murphy, JR.
Reg. No. 28,977

MaryAnne Armstrong, Ph.D.
Reg. No. 40,069

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

GMM/MAA/

MARKED-UP VERSION SHOWING CHANGES

Claims 1 and 17 have been amended as follows.

1. (twice amended) A method for generating artificial SH3 domains having desired ligand binding properties **and screening the domains for desired ligand binding properties**, which comprises:

a) producing a collection of DNA fragments encoding SH3 domains containing a randomized RT-loop (RRT-SH3 domains),

b) generating recombinant libraries comprising said RRT-SH3 domains, and

c) subjecting said libraries to affinity or functional selection steps to ~~generate~~ **identify** artificial SH3 domains.

17. (amended) The method according to claim 3, wherein the six amino acids that are replaced in the RT-loop are replaced with a peptide motif **derived from Hck-SH3 and targeted to the HIV-1 Nef protein** selected from the group consisting of XSWSXX (SEQ ID NO:28), XSPFXX (SEQ ID NO:30) and XSXFPW (SEQ ID NO:32), wherein X is any amino acid.